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Increased Collagen, Per Se, May Not Affect Left Ventricular Function in Spontaneously Hypertensive Rats

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ABSTRACT

Background: Left ventricular fibrosis is considered to be a major participant in the development of cardiac dysfunction in various conditions (hypertension, aging, etc). Because cardiac myocytes as well as blood supply may also be affected in these conditions, it is difficult to define quantitatively the role of fibrosis. We hypothesized that by inducing myocardial collagen accumulation by treatment with an inhibitor (doxycycline) of matrix metalloproteinases, which by itself should not affect cardiac myocytes, we might examine a more specific role of fibrosis in cardiac dysfunction.

Methods: Adult male spontaneously hypertensive rats were divided into 2 groups. The control group received no treatment; the second group was given doxycycline (30 mg/kg/day) for 6 months. Arterial pressure, pulse wave velocity, indexes of heart function (end-diastolic pressure, maximal rates of pressure rise and fall [dP/dt_{max} and dP/dt_{min}], diastolic time constant [Tau]), weight indexes, and myocardial collagen concentration were determined at the end.

Results: The results demonstrated that treatment with an inhibitor of matrix metalloproteinases induced significant accumulation of ventricular collagen, as indicated by increased ventricular hydroxyproline concentration (4.71 $\pm$ 0.12 mg/g vs 5.35 $\pm$ 0.17 mg/g in control and doxycycline groups, respectively). However, arterial pressure, aortic stiffness (pulse wave velocity), and left ventricular function were unaffected.

Conclusions: These findings suggest that moderate collagen accumulation does not by itself adversely affect cardiovascular function and that other changes in collagen properties (eg, formation of advanced glycation end-products) may be responsible for the adverse effects of myocardial fibrosis.

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INTRODUCTION

Ventricular extracellular matrix and its most abundant components, collagen types I and III, provide structural integrity to the heart muscle and contribute importantly to ventricular pump function through the coordination of myocyte contraction.[1-3] The structural and functional integrity of normal myocardium is maintained by a balanced equilibrium between its components, including myocytes and nonmyocytic cells (endothelial and vascular smooth muscle cells of the intramural coronary circulation and fibroblasts) and all components of extracellular matrix.[2-4] Thus, under normal physiological conditions, collagen synthesis and degradation are in equilibrium in myocardial tissue; therefore, the collagen concentration remains fairly constant. However, in a number of cardiac disorders, including hypertensive and ischemic heart disease, as well as cardiomyopathies, the balance between synthesis and degradation of collagen is disrupted, leading to accumulation of collagen and ventricular fibrosis of varying degree.[4,5] This disequilibrium does not result merely from an alteration to one side of the process but rather from a complex interplay of biological changes involving both synthesis and degradation.[4,5] This myocardial fibrosis alters myocardial stiffness and consequently affects myocardial function.[4-6] However, because in addition to accumulation of collagen the cardiac myocytes are also affected in various cardiac disorders, it is difficult to define precisely the role of fibrosis in the development of left ventricular (LV) dysfunction and heart failure in quantitative terms.

Myocardial collagen is degraded by a number of proteases, commonly known as matrix metalloproteinases.[1,4] Thus, we hypothesized that it may be possible to induce myocardial collagen accumulation by treatment with a nonselective inhibitor (doxycycline) of matrix

metalloproteinases and that this process, by itself, should not affect the function of cardiac myocytes. Consequently, this experimental model should permit closer examination of a specific role of increased myocardial collagen content, per se, on LV function. To this end, spontaneously hypertensive rats (SHRs) were given either placebo or doxycycline. Because the half-life of collagen type 1, which is dominantly expressed in the hypertrophied left ventricle of SHRs, is approximately 100 days, we extended this treatment to 6 months.[2]

MATERIALS AND METHODS

Experimental Animals

The 24 male SHRs, purchased from Harlan Laboratories (Indianapolis, IN), were maintained in a temperature- and humidity-controlled room with a 12-hour light/dark cycle. All rats were permitted free access to their chow and tap water. Rats were handled in accordance with National Institutes of Health guidelines, and our Institutional Animal Care and Use Committee approved the study protocol in advance.

Experimental Protocol

Male, 20-week-old SHRs were divided into 2 groups (12 rats in each). The control group received no treatment other than their standard chow and tap water; the second group was given a nonspecific inhibitor of matrix metalloproteinases, doxycycline (30 mg/kg/day), for 6 months. This dose of doxycycline has been previously shown to increase ventricular collagen content in rats.[7] Arterial pressure, LV function, cardiovascular mass indexes, and the extent of cardiac fibrosis were examined at the end of the 6-month course of the experiment. To this end, all rats were anesthetized with pentobarbital (40 mg/kg, intraperitoneally), and the right carotid artery was cannulated with a transducer-tipped catheter (Micro-Tip 3F, Millar Instruments, Houston, TX) that was advanced into the ascending aorta for recording of arterial pressure. A second catheter was placed into the abdominal aorta through the femoral artery. Both arterial catheters were connected to a multichannel recorder (Grass Technologies, West Warwick, RI) interfaced to an IBM computer with a digital data acquisition system (EMKA Technologies, Falls Church, VA). To determine pulse-wave velocity (PWV), pulse contours from the 2 catheters were registered simultaneously on the same channel.[8,9] The PWV was calculated as the aortic length between the 2 catheters (measured postmortem) and the time difference between their diastolic notches. After aortic functional measurements were made, the catheter already placed in the ascending aorta was advanced further into the left ventricle. The maximal and end-diastolic pressures in the left ventricles, as well as the first derivatives of pressure over time (dP/dt_{max} and dP/dt_{min}) and diastolic time constant (Tau) as indexes of global contractility and relaxation, were recorded.[8-10]

At the end of the study, the rats were killed with an overdose of pentobarbital, and their hearts, aortas, and kidneys were removed and weighed. As an estimate of ventricular collagen content, hydroxyproline concentration in the samples from both ventricles was determined.[9,10] Briefly, myocardial samples (approximately 100 mg) were weighed and dried overnight in a 60°C oven to constant weight. Lipids were then extracted twice with a 2:1 mixture of chloroform and methanol. Fat-free samples were dried overnight at 60°C and weighed. Collagen was hydrolyzed at 110°C overnight into its component amino acids with 6N hydrochloric acid. After extraction with activated charcoal, the samples were treated with chloramine T and paradimethylaminobenzaldehyde solution. Hydroxyproline concentration was measured spectrophotometrically (DU640B Spectrophotometer, Beckman Coulter, Brea, CA) at 560 nm. Hydroxyproline concentration was expressed as mg/g of dry weight.[9,10]

Statistical Analysis

All values are expressed as the mean ±1 standard error of the mean. Data were analyzed by unpaired t-test.[11] A value of $P < .05$ was considered to be statistically significant.

RESULTS

Body Weights and Organ Weight Indexes

We observed no difference in body weight between the 2 groups (412 ± 9 g vs 418 ± 11 g in control group and doxycycline group, respectively). Similarly, we saw no differences in aortic weight index (1.38 ± 0.06 mg/mm vs 1.42 ± 0.04 mg/mm) or kidney weight index (4.12 ± 0.12 mg/g vs. 3.98 ± 0.13 mg/g) between the control rats and the doxycycline-treated rats, respectively.

Cardiac Weights and Collagen Content

We found no differences between the LV and right ventricular weights of the control and doxycycline groups (Figure 1). However, when compared with their respective controls, collagen content, as determined by hydroxyproline concentration, was significantly greater in both ventricles of doxycycline-treated rats (Figure 1).

Arterial Pressure and Heart Rate

The systolic, diastolic, and mean arterial pressures, as well as heart rate, were similar between the groups (Figure 2).

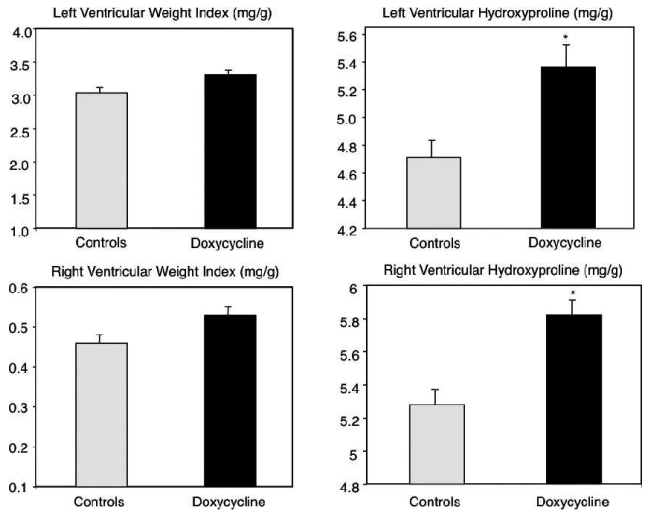

Figure 1. Left and right ventricular weight indexes and hydroxyproline concentration in control and doxycycline-treated rats. Values are mean ± 1 standard error of the mean. *P* < .05 when compared to control. Twelve animals per group.

Pulse Wave Velocity

We discovered no differences in PWV, either absolute or normalized for diastolic pressure (as an estimate of a degree of aortic stretch), between the groups (Figure 3).

Indexes of Left Ventricular Function

LV end-diastolic pressure, maximal rates of ventricular pressure rise and decline, and diastolic time constant were similar in the control and doxycycline-treated rats (Figure 4).

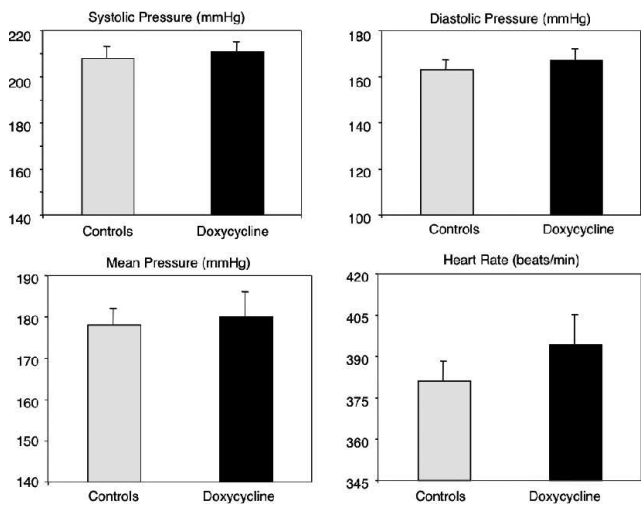

Figure 2. Systolic, diastolic, and mean arterial pressure and heart rate in control and doxycycline-treated rats. Values are mean ± 1 standard error of the mean. *P* < .05 when compared to control. Twelve animals per group.

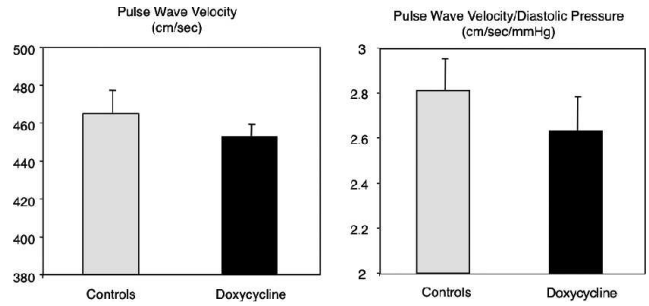

Figure 3. Pulse wave velocity and pulse wave velocity normalized for diastolic pressure in control and doxycycline-treated rats. Values are mean ± 1 standard error of the mean. *P* < .05 when compared to control. Twelve animals per group.

DISCUSSION

This study demonstrated that chronic intervention with a matrix metalloproteinases inhibitor, doxycycline, induced moderate accumulation of collagen in both ventricles of the SHRs, as indicated by increased hydroxyproline concentrations in myocardial tissue. This finding is in agreement with previous reports on the effects of doxycycline on collagen accumulation in the rat heart and subcutaneous granulomas.[7,12] However, there were no effects of doxycycline on arterial pressure, PWV, or ventricular function. Thus, the data derived from this study suggest that moderate collagen accumulation does not by itself adversely affect cardiovascular function. Yet, this finding does not rule out the possibility that changes in collagen properties (eg, extent of cross-linking or formation of advanced glycation end-products [AGEs]) may be responsible for the adverse effects of ventricular fibrosis. In addition, the solitary change in ventricular fibrosis does not relate to the status of ventricular blood flow, associated apoptosis, or other coincidental biological changes.

However, this conclusion is at variance with the findings of previous studies showing that ventricular fibrosis alters myocardial stiffness and consequently may affect myocardial function.[4-6,13-15] Actually, researchers generally agree that extracellular matrix remodeling of the ventricle may exert a profound effect on diastolic function.[5,6,14,16,17] Several possible explanations exist for this divergence between our results and findings of the aforementioned studies. First, it may be a matter of collagen quantity. When compared to control rats, an increased hydroxyproline concentration of about 10% was observed in doxycycline-treated SHRs in the present study. In previous studies from our laboratory showing a good correlation between ventricular collagen content and ventricular function, there was at least a 25% increase

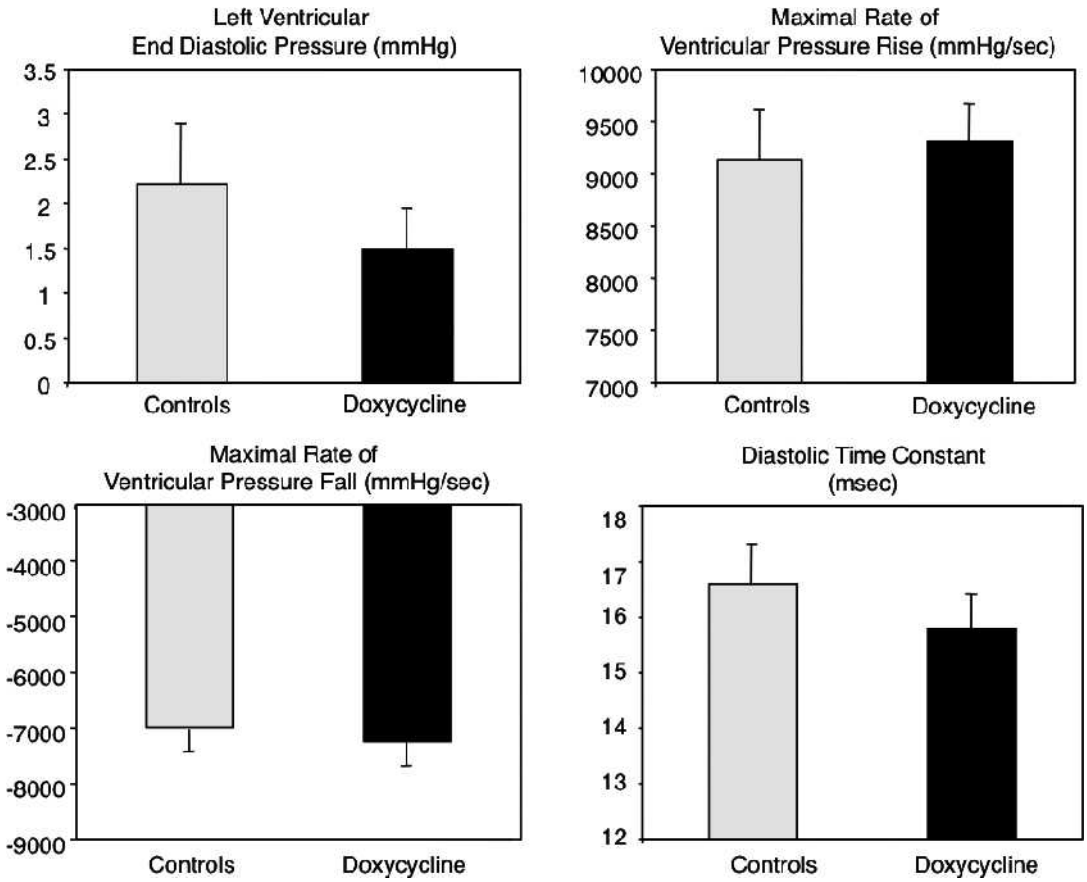

Figure 4. Indexes of heart function in control and doxycycline-treated rats. Values are mean ± 1 standard error of the mean. $P < .05$ when compared to control. Twelve animals per group.

in ventricular hydroxyproline.[8,18-20] Therefore, it is possible that the modest increase in ventricular collagen in this report was insufficient to affect ventricular function adversely. It is also possible that the indexes of ventricular function determined in this study were not sensitive enough to detect slight functional changes. Thus, maximal rate of LV pressure decline and diastolic time constant were used as estimates of diastolic function, and both of these indexes were better suited to estimate the effectiveness of the active phase of diastolic relaxation (ie, myocyte relaxation and calcium turnover) than myocardial stiffness. It may not have been possible to detect slight changes in diastolic relaxation. However, any significant increase in ventricular stiffness might have resulted in increased end-diastolic pressure that was not observed in this study. Finally, it is also possible that it is not the absolute amount of collagen that mediates adverse cardiovascular effects of fibrosis, but rather other properties of collagen may be of importance.

It has been shown that increased myocardial stiffness in hypertensive rats was the consequence of enhanced myocardial collagen cross-linking rather than of an increase in total collagen of collagen type I to III ratio.[21,22] It is also possible that formation of AGEs is responsible for increased collagen and hence myocardial stiffness. Thus, it is well established that prevention of AGE formation, as well as the breaking of already formed cross-links, significantly improves diastolic function in hypertensive and aging animals.[23-25]

Although the possible role of collagen in affecting LV diastolic function is known, a definite cause and effect relationship between excessive collagen accumulation and systolic function has not been established.[26-28] Furthermore, evidence indicates that reduction in the myocardial collagen matrix could exacerbate the severity of heart failure.[26] Similarly, research has shown that reducing ventricular collagen decreased systolic performance without any effect on diastolic function in hypertensive rats.[27] It is also of note that abnormalities in the collagen scaffold could facilitate alterations in the physiological orientation of the muscular fibers, resulting in the impairment of transmission of contractile force through the myocardial wall.[28] In addition, some have suggested that the architecture of the scaffold plays a significant role in determining ventricular function.[28]

In conclusion, the presented results suggest that a modest increase in myocardial collagen does not per se affect LV function and that changes in collagen properties (extent of collagen cross-linking, formation of AGEs) may be responsible for adverse cardiovascular effects of ventricular fibrosis. Further detailed studies of the mechanism of deleterious effects of cardiac fibrosis should follow. Finally, pathological studies have demonstrated that the adverse effects of collagen deposition in the ventricles are also associated with ischemia, apoptosis, and other alterations.[6,17]

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